BASIC RESEARCH STUDIES

From the Midwestern Vascular Surgical Society

Response of plasma matrix metalloproteinase–9 to conventional abdominal aortic aneurysm repair or endovascular exclusion: Implications for endoleak

David R. Lorelli, MD, Jessie M. Jean-Claude, MD, Christopher J. Fox, MD, Jason Clyne, BS, Robert A. Cambria, MD, Gary R. Seabrook, MD, and **Jonathan B. Towne, MD,** *Milwaukee, Wis*

Purpose: **Matrix metalloproteinases are enzymes capable of breaking down all of the components of the extracellular matrix and have been implicated in the development of aneurysm formation. Because matrix metalloproteinase–9 (MMP-9) levels are elevated in aortic aneurysmal tissue and in that patient plasma, we hypothesized that plasma MMP-9 levels should decrease significantly after conventional and endovascular infrarenal abdominal aortic aneurysm (AAA) repair but that plasma MMP-9 levels would remain elevated in patients with endoleaks.**

Methods: **A sandwich enzyme-linked immunosorbent assay was used to measure plasma levels of MMP-9 in patients with AAA who underwent conventional (n = 26; mean age, 71.5 years) and endovascular (n = 25; mean age, 76.4 years) AAA repair. Levels were drawn before surgery and at 1 month and 3 months after surgery. Eight patients for endovascular repair had endoleaks identified on postoperative computed axial tomographic scans.**

Results: **No correlation existed between preoperative plasma MMP-9 levels when compared with age, gender, or aneurysm diameter. No significant difference in preoperative plasma MMP-9 levels or AAA diameter was identified between patients with conventional repair compared with endovascular repair. Of the 51 patients, 33 had follow-up samples available for analysis. A significant increase in mean plasma MMP-9 levels was noted 1 month (149.5 ± 40.1 ng/mL) after conventional AAA repair compared with preoperative levels (83.9 ± 26.1 ng/mL; $P < .05$) and remained elevated 3 months after surgery (129.8 ± 56.6 ng/mL). In those patients who underwent endovascular aneurysm exclusion without endoleak, a significant decrease in mean plasma MMP-9 levels was noted at 3 months (27.4 ± 5.2 ng/mL) when compared with preoperative values (60.8 ± 8.8 ng/mL; $P < .01$). In contrast, patients with endoleak after endovascular exclusion did not have a significant decrease in plasma MMP-9 levels at 3 months.**

Conclusion: **Plasma MMP-9 levels remain elevated for as much as 3 months after conventional AAA repair, whereas successful endovascular exclusion of an AAA results in decreased plasma MMP-9 levels by 3 months. MMP-9 may have clinical value as an enzymatic marker for endoleak after endovascular AAA exclusion. (J Vasc Surg 2002;35:916-22.)**

In the past 50 years, extraordinary strides have been made in understanding the pathogenesis of aneurysmal disease and in its surgical treatment with the advent of endovascular stent grafting.[1] As the understanding of aortic aneurysmal disease becomes more sophisticated, vascular surgeons are using new techniques to reduce the morbidity and mortality rates associated with aneurysm rupture and to avoid complications associated with the procedure.

From the Division of Vascular Surgery, Medical College of Wisconsin.
Supported by the E. R. Wagner Fund.
Competition of interest: nil.
Presented at the Twenty-fifth Annual Meeting of the Midwestern Vascular Surgical Society, Chicago, Ill, Sep 21-22, 2001.
Reprint requests: Jonathan B. Towne, MD, Chairman, Vascular Surgery, Medical College of Wisconsin, 9200 West Wisconsin Ave, Milwaukee, WI 53226 (e-mail: jtowne@mcw.edu).

0741-5214/2002/$35.00 + 0 **24/6/123676**
doi:10.1067/mva.2002.123676

Collagen and elastin play important roles in the structure and function of the aorta.[2] Matrix metalloproteinases are soluble, zinc-dependent enzymes produced by macrophages and smooth muscle cells that are capable of breaking down components of the extracellular matrix and have been implicated by many investigators in the development of aneurysm formation.[3-6] Matrix metalloproteinase-9 (MMP-9) has elastolytic activity that may act in concert with proteases and other metalloproteinases to affect structural arterial changes in aneurysmal disease.[7] Elevated MMP-9 levels in aortic aneurysmal tissue, and in that patient plasma, have been shown.[8] This increased enzyme level has been implicated in the progression of aneurysmal disease.

Since its introduction in 1991, endovascular abdominal aortic aneurysm (AAA) repair has been shown to be a viable alternative to open repair in short to medium term follow-up periods. Compared with conventional repair, endovascular repair leaves the aneurysm sac in situ and undis-

turbed. After exclusion, thrombosis and regression of the aneurysm sac are expected. However, in a subset of patients, an endoleak persists or develops that continually exposes the aneurysm sac to arterial pressure and blood flow. Stent migration or graft component separation can lead to type I endoleak. Although the exact association between a type II endoleak and the risk for aneurysm rupture is not known, because the sac remains under pressure, the potential for growth and rupture still exists. Type II endoleaks commonly occur via patent lumbar or inferior mesenteric arteries. Indeed, at least 25 cases of aneurysm rupture after endovascular repair have been reported to the Food and Drug Administration.[9] Detection and treatment of endoleak is, therefore, crucial to successful endovascular aneurysm repair. Thus, a biologically relevant marker of aneurysm disease and its trend after aneurysm repair would be valuable in identifying these patients at risk of aneurysm rupture. Diagnosis has depended on computed tomographic (CT) scanning to visualize flow in the aneurysm sac. However, the sensitivity of this imaging method is not 100%.[10,11] Excluded aneurysms have continued to enlarge without demonstrable flow in the aneurysm sac and consequently are still at risk for rupture.[11]

This study suggests that plasma levels of MMP-9 can be used as a marker for persistent endoleak in those patients who undergo endovascular repair of an AAA. The specific aims of the study were to determine whether plasma MMP-9 levels drop significantly after conventional and endovascular AAA repair and to determine whether patients with persistent endoleaks after endovascular repair have persistently elevated plasma MMP-9 levels. To date, we are unaware of any study that has compared the response of plasma MMP-9 with conventional AAA repair and endovascular exclusion or has investigated its implications for the diagnosis of endoleak.

METHODS

All patients who underwent elective infrarenal AAA repair between September 2000 and April 2001 were eligible for study inclusion. Informed consent was obtained in accordance with the Institutional Review Board of the Medical College of Wisconsin and the Veterans Administrations Medical Center in Milwaukee. Fifty-one patients were enrolled in the study. Excluded were those patients who were unwilling to participate and those with aneurysm rupture.

Conventional aneurysm repair implanted Dacron aortic interposition or bifurcated grafts, handsewn with prolene suture. The residual aneurysm sac was closed around the aortic graft. Endovascular aneurysm exclusion was accomplished with the two commercially available (AneuRx, Medtronics, Santa Rosa, Calif, and Ancure, Guidant, Menlo Park, Calif) endovascular stent graft systems. A custom-made Malmo-Ivancev stent graft also was used as part of an Investigational Device Exemption granted to a senior staff member from the Food and Drug Administration. This is a fully supported aortomonoiliac device consisting of a noncrimped Dacron graft (LeMaitre Vascular Inc, Burlington, Mass) handsewn to a tapered Gianturco stent (Cook Inc, Bloomington, Ind) with Gore Tex (W.L. Gore, Flagstaff, Ariz) sutures. The contralateral iliac artery was occluded, and revascularization was performed with a femoral-femoral bypass graft. Patients for commercial endovascular procedures underwent surveillance with abdominal CT scans at 1 month and 3 months after surgery, and those with the custom device underwent imaging before discharge and then again at 3 months.

Blood was collected from venipuncture or central venous catheters into tubes containing sodium ethylenediamine tetraacetate. Samples were obtained before surgery on the morning of the procedure in the preoperative area after central venous access was obtained and at 1 and 3 months after surgery. The blood samples were centrifuged ($1000 \times g$ for 15 minutes) within 45 minutes of collection. The plasma obtained from the samples was frozen at $-84°$ C and stored until use. A commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kit with an MMP-9 monoclonal antibody (Biotrak MMP-9 Human ELISA System, Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom) was used to determine plasma MMP-9 levels. A log-log linear regression curve of purified standards was used to extrapolate and quantify the individual plasma MMP-9 level. All plasma samples were assayed with an initial 1:10 dilution. Spectrophotometric density measurements were made at 450 nm with an automated microplate reader (Bio-Tek Instruments, Winooski, Vt). Samples whose plasma value fell outside of the linear range of the ELISA (4 to 128 ng/mL) were sequentially diluted and the assay was repeated. All the samples were run in duplicate, and an average value was obtained. If a 10% or greater difference in measured MMP-9 values was encountered, then that particular sample was run in triplicate to determine an average of the three values. The ELISA was able to measure all forms of pro–MMP-9, including the pro–MMP-9/tissue inhibitor of the metalloproteinases-1 (TIMP-1) complex. According to the manufacturer, the specificity of the assay reveals no detectable cross-reactivity with MMP-1, MMP-2, MMP-3, TIMP-1, or TIMP-2. The within-assay precision (<6%) and the between assay precision (<10%) were determined by the manufacturer.

The data were recorded as the mean ± the standard error of the mean and compared with a t test for paired samples. Analysis was performed with log means for normality because of nonnormal distribution of values. Linear regression analysis was performed in comparing aneurysm size and preoperative plasma levels. Statistical comparisons between groups in regards to patient characteristics of age, gender, history of hypertension on the basis of use of antihypertensive agents, diabetes on the basis of the use of insulin or oral hypoglycemic agent, and any history of smoking was performed with χ^2 analysis.

RESULTS

Preoperative plasma MMP-9 levels were available in 51 patients, 26 who underwent conventional aneurysm repair

Patient and AAA characteristics

	Conventional	*Endovascular*	P *value*
No. of patients	26	25	
Age (y)	71.5 ± 1.4	76.4 ± 1.5	<.05
Sex (male:female)	19:7	22:3	NS
Hypertension	65%	80%	NS
Diabetes	15%	8%	NS
Tobacco use	69%	64%	NS
AAA diameter (mm) (range; mm)	59.1 ± 2.1 (46-100)	57.5 ± 1.8 (47-74)	NS
Preoperative MMP-9 (ng/mL) (range; ng/mL)	70.2 ± 16.8 (2.8-402.4)	56.3 ± 7.5 (7.7-140.1)	NS

NS, Not significant.

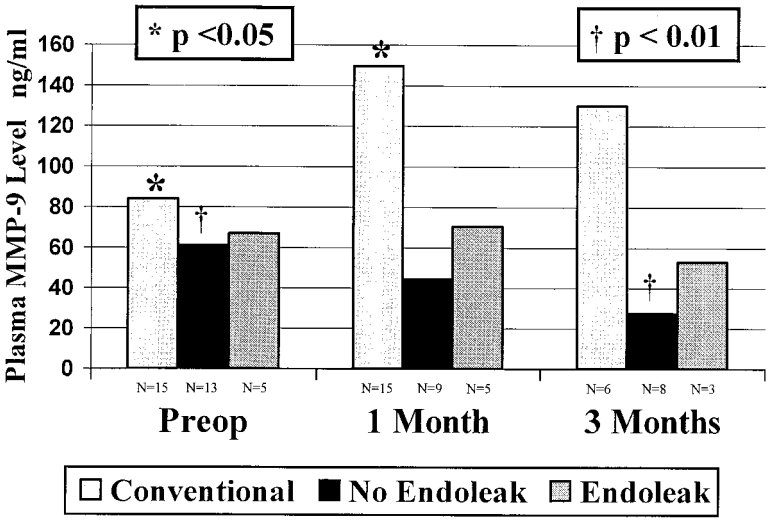

Fig 1. Comparison of mean plasma MMP-9 levels (ng/mL) over time between groups. *N* denotes number of patients in each category.

and 25 with endovascular exclusion. The demographics and comorbidities of both groups are listed in the Table.

Conventional aneurysm repair used 16 bifurcated and 10 interposition Dacron grafts. Only one patient was known to have a small residual iliac aneurysm after aortic aneurysm resection was completed. Of those patients who underwent endovascular repair, 16 were treated with the Ancure device, six were treated with the AneuRx device, and three patients were treated with the Malmo-Ivancev stent graft. Those patients who underwent treatment with conventional open repair were younger when compared with their endovascular counterparts (71.5 ± 1.4 years versus 76.4 ± 1.5 years; $P < .05$). Neither a positive nor negative correlation existed between preoperative plasma MMP-9 levels when compared with age, gender, or aneurysm diameter. No significant difference existed ($r = 0.13$; $P =$ not significant) in mean preoperative MMP-9 levels when classifying aneurysm size into small (≤49 mm; 58.9 ± 12.8 ng/mL), medium (50 to 69 mm; 64.8 ± 11.8 ng/mL), and large (≥70 mm; 59.4 ± 17.2 ng/mL) diameters. No significant difference in preoperative plasma MMP-9 levels, nor aneurysm diameter, could be identified between patients with conventional repair compared with endovascular repair (Table).

Postoperative plasma samples were available for analysis in 15 of 26 patients for conventional repair and in 18 of 25 patients for endovascular repair. Endoleak was evident on follow-up CT scan in 32% (8/25) of the endovascular group. In five of the eight patients with endoleaks, samples were available for follow-up analysis. Mean plasma MMP-9 levels peaked at 1 month (149.5 ± 40.1 ng/mL) when compared with preoperative levels (83.9 ± 26.1 ng/mL; $P < .05$) and remained elevated (129.8 ± 56.6 ng/mL) 3 months after conventional AAA repair (Fig 1). In those patients who underwent endovascular exclusion without endoleak, a significant decrease in plasma MMP-9 levels was noted between preoperative and 3-month levels (60.8 ± 8.8 ng/mL versus 27.4 ± 5.2 ng/mL; $P < .01$). In contrast, patients with endoleak after endovascular exclusion did not have a significant decrease in plasma MMP-9 levels at 3 months (67.1 ± 26.7 ng/mL versus 53.0 ± 11.2 ng/mL; $P =$ not significant). One patient had

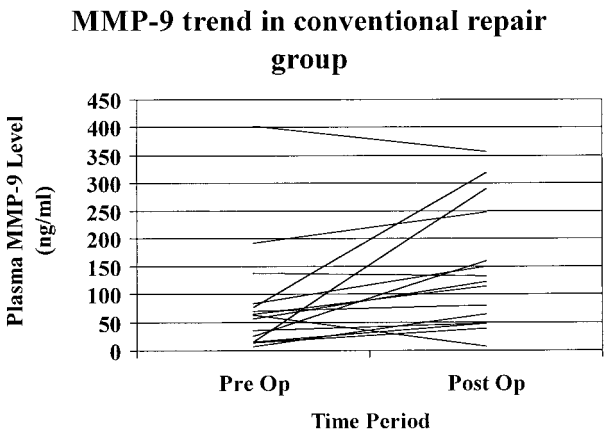

Fig 2. Plot of MMP-9 changes in conventional repair group.

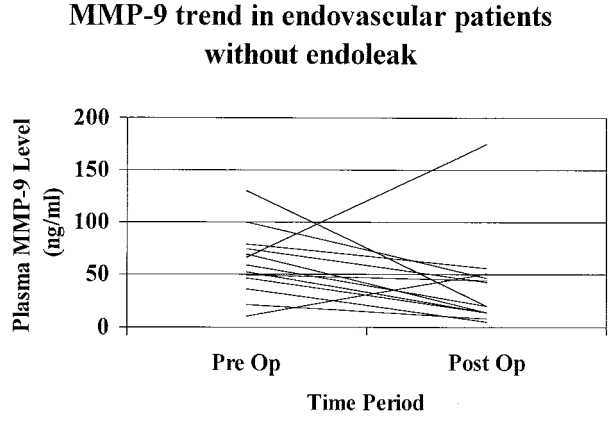

Fig 3. Plot of MMP-9 changes in endovascular group without endoleak.

a proximal attachment site (type I) endoleak (Ancure), and seven of the endoleaks were type II in origin (Ancure = 5, AneuRx = 1, Malmo-Ivancev = 1). Seven of the patients with endoleaks had aneurysm sizes that were unchanged at 3 months, and one patient actually had a decrease in the size of the AAA.

Analysis of the direction of change in the plasma MMP-9 levels revealed that follow-up values at 1 or 3 months were increased an average of 63% (mean change, +65 ng/mL) in 12 of 15 patients (80%) who underwent conventional AAA repair (Fig 2). For 11 of 13 patients (85%) with successful endovascular AAA repair (Fig 3), MMP-9 levels were decreased an average of 35% (mean change, −19 ng/mL). In contrast, MMP-9 levels were increased an average of 15% (mean change, +3 ng/mL) for four of five patients (80%) with endoleak after endovascular repair (Fig 4).

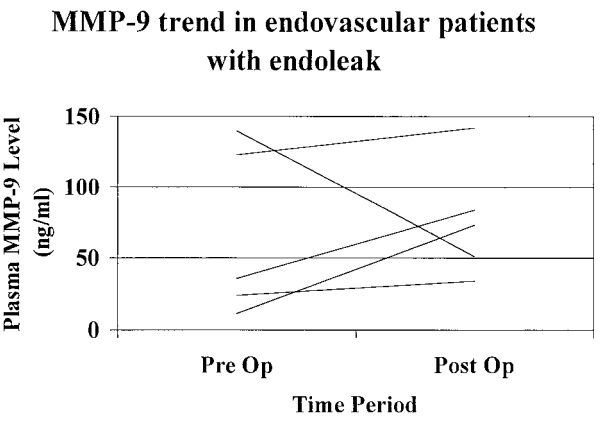

Fig 4. Plot of MMP-9 changes in endovascular patients with endoleak.

DISCUSSION

Investigations of aneurysms have sought to identify a biologically relevant marker of the disease process.[8,12-14] Since Busuttil et al[15] described the role of elastase in aneurysm formation and later characterized a soluble metalloelastase,[3] significant progress has been made in isolating and describing matrix metalloproteinases and in elucidating their role in the development of arterial aneurysms.[16-18] MMP-9 has been shown to be the principle elastase within the aneurysmal aortic wall and is expressed by macrophages in excess when compared with other metalloproteinases.[8,19] This overproduction of MMP-9, induced by a chronic inflammatory state, contributes to the progression of aneurysmal disease by mediating the connective tissue destruction seen in the extracellular matrix.[20] Indeed, experimental models have shown that inhibition of these proteinases may actually slow matrix breakdown and limit the growth of aneurysms.[21-26] Although plasma levels of MMP-9 may not be accurate enough for use as a diagnostic screening test of AAA,[20] MMP-9 may have a role in evaluation of patients with known aneurysmal disease and in the response to endovascular or pharmacologic treatment.[20]

The elevated plasma MMP-9 levels in patients with aortic aneurysm in this study is similar to the levels in the previous studies of McMillian and Pearce[8] (85.7 ± 11.6 ng/mL; range, 21.2 to 203.8 ng/mL) and Hovsepian et al[20] (99.4 ± 17.4 ng/mL; range, 16.3 to 402.6 ng/mL). This compares with values of 13.2 ± 1.9 ng/mL (range, 7.1 to 21.1 ng/mL) and 36.1 ± 7.7 ng/mL (range, 14.0 to 62.4 ng/mL), respectively, for healthy control subjects in these previous two studies.[8,20] Despite a significant difference in age between the two groups (Table), no correlation could be found with preoperative plasma MMP-9 levels and age of the patient. A similar finding was reported by Hovsepian et al.[20] These data reveal no difference in MMP-9 levels on the basis of gender, which other investigators also have shown.[8,20] Likewise, neither a pos-

itive nor a negative correlation was detected for plasma MMP-9 levels and aneurysm diameter, which ranged from 46 to 100 mm. This included analysis after stratification into small (diameter, ≤49 mm), moderate (diameter, 50 to 69 mm), and large aneurysms (diameter, ≥70 mm). Our findings are in contradistinction to the findings of McMillian et al[27] who showed a four-fold to five-fold elevation in MMP-9 messenger RNA expression with moderate-sized aneurysms compared with either small or large ones. This difference is thought to relate to increased elastolytic activity in moderate-sized aneurysms, with great variability corresponding to the rate of aneurysm expansion.[27] The decreased levels found in large aneurysms may relate to decreased numbers of inflammatory cells available to produce MMP-9. In addition, elastase may be depleted in larger aneurysms, as may be the relative number of smooth muscle cells.[28] The expansion of large aneurysms may have more to do with mechanical wall forces than enzymatic matrix destruction.[27] Although mean preoperative plasma MMP-9 levels were slightly higher for moderately sized aneurysms when compared with small or large aneurysms in this study, the difference was not significant.

Why considerable variability exists in preoperative plasma MMP-9 levels in this study, or in other studies, is unclear.[8,20] MMP-9 levels may better correlate with the extent of aneurysmal disease as opposed to aneurysm diameter. Indeed, increased enzyme levels were significantly associated with the presence of multiple aneurysms in a study of 22 patients with aneurysm.[8] In this study, three patients had a history of multiple aneurysms. However, no obvious or significant differences in plasma MMP-9 levels compared with the rest of the cohort existed. This lack of difference may mask a type II error because of small group size. In addition, preoperative MMP-9 levels may reflect a current rate of aneurysm expansion.[27] Patients referred for surgical evaluation usually have aneurysms large enough to necessitate intervention. Thus, the opportunity to correlate expansion with MMP-9 activity in a surgical series is limited and would more appropriately be undertaken in conjunction with screening initiatives, which can identify small aneurysms and document the rate of expansion before reaching a size necessitating operative or endovascular intervention.

For those patients who underwent conventional aneurysm repair, these short-term follow-up data revealed a significant increase in mean plasma MMP-9 levels at 1 month that remained elevated at the 3-month time period. Because standard aneurysm resection removes all diseased aortic wall from the hemodynamic effects of the circulation, theoretically plasma MMP-9 levels should have reduced dramatically. Although zymography was not performed to determine whether the plasma MMP-9 was functionally active, we concur with many studies that have shown the diseased aortic wall to be the source of metalloproteinase production.[29-32] The enzyme is produced as a result of a cytokine-mediated chronic inflammatory state of unclear cause, which is responsible for macrophage infiltration of the aortic wall. The physiologic stress of an open aneurysm repair possibly may itself induce an acute inflammatory response or upregulate the chronic inflammatory response such that plasma levels of MMP-9 are actually increased for as much as 3 months. Indeed, other investigators have shown elevated levels of proinflammatory cytokines, such as tumor necrosis factor–α, C-reactive protein, interferon-γ, interleukin-1, and interleukin-6.[33-36] Alternatively, manual manipulation of the aneurysm sac during open repair may somehow contribute to elevated levels in the short term. The only other study to have evaluated MMP-9 levels after open aneurysm repair in six patients showed that MMP-9 levels decreased by an average of 92%.[20] However, five of the six patients had follow-up samples taken between 6 and 10 months after surgery. If the acute elevation shown at 3 months in this study results from an altered inflammatory response, the levels might be expected to decrease after 6 months. In addition, the residual aneurysm wall is routinely wrapped around the aortic graft and remains perfused and viable. This tissue may take months to eventually scar and plasma MMP-9 levels to decline. Indeed, two of the patients with conventional repair had follow-up MMP-9 levels evaluated at 6 months, and the values decreased from preoperative levels by 40% and 42%. In addition, three patients in the conventional repair group had infectious or inflammatory complications, postoperative diverticulitis, clostridium difficile colitis, and a groin wound infection, which may have contributed to elevated postoperative MMP-9 levels.

In comparison of patients with conventional repair with those who underwent endovascular repair without evidence of endoleak, these patients actually had a significant decrease in mean plasma MMP-9 levels at 3 months. These patients had a less invasive surgical procedure without aortic cross clamping and with minimal physiologic disturbance, which may not elicit a generalized inflammatory response compared with those who underwent laparotomy. In addition, the large degree of destruction and manipulation of the aneurysm sac does not occur during endovascular exclusion, and both of these factors may account for the decrease in enzyme levels at 3 months. None of the 13 patients who underwent endovascular repair had elevated MMP-9 levels in the absence of an endoleak on the initial postoperative study, and none had an endoleak develop on later imaging. In addition, no correlation could be found between changes in plasma MMP-9 levels and changes in AAA sac diameter after endovascular repair. Changes in aneurysm volume were not evaluated. In contrast, MMP-9 levels did not significantly decrease at 3 months in patients with endoleak after endovascular repair. Our patient numbers were too small to make meaningful distinction between the different types of endoleak. Of note, the patient with single endoleak with decreasing MMP-9 levels had a type II endoleak from a patent inferior mesenteric artery and had recently stopped smoking before surgery. In addition, because the follow-up period was short, none of these patients had undergone any type of intervention to treat the endoleak. Theoretically, patients with endoleak still have an aneurysm exposed to the systemic circulation, and there-

fore, the biologic milieu of the aneurysm would not be significantly altered. This persistence of plasma MMP-9 levels becomes clinically relevant in evaluation of individual patients after endovascular exclusion. Although great variation exists in absolute preoperative MMP-9 levels, classification of plasma levels as normal or abnormal for an individual patient is difficult.[20] However, the direction of change in the plasma enzyme level may be beneficial to the clinician. These data reveal that quantification of plasma MMP-9 levels may serve as an enzymatic marker for endoleak after endovascular exclusion.

This study has shown that plasma MMP-9 levels remain elevated after conventional AAA repair for as much as 3 months and that successful endovascular exclusion results in decreased plasma MMP-9 levels in the same interval. Patients with endoleak after endovascular exclusion tend to have plasma MMP-9 levels that do not significantly change from preoperative levels. One may speculate that the change in plasma MMP-9 level might be a better indicator of the biologic significance associated with an endoleak than characteristics assessed with radiographic imaging alone. An endoleak associated with a decrease in plasma MMP-9 level might be of less significance than one in which MMP-9 level had remained elevated. Such trends are valuable considerations for future study. We currently do not have long-term follow-up data available for these patients. Patients with conventional treatment with persistently elevated levels of MMP-9 may be predisposed to development of suprarenal, iliac, or popliteal aneurysms. In addition, as our experience with endovascular exclusion of aneurysms grows, evaluation of MMP-9 levels in patients with endoleaks who subsequently undergo intervention will merit study.

We thank Raymond G. Hoffman, PhD, Division of Biostatistics, for his assistance in statistical analysis and Dr Krasnodar Ivancev for the use of his custom-made aortic stent graft device.

REFERENCES

1. Friedman SG. The 50th anniversary of abdominal aortic reconstruction. J Vasc Surg 2001;33:895-8.
2. Rehm JP, Grange JJ, Baxter BT. The formation of aneurysms. Semin Vasc Surg 1998;11:193-202.
3. Brown SL, Backstrom B, Busuttil RW. A new serum proteolytic enzyme in aneurysm pathogenesis. J Vasc Surg 1985;2:393-9.
4. Ghorpade A, Baxter BT. Biochemistry and molecular regulation of matrix macromolecules in abdominal aortic aneurysms. Ann N Y Acad Sci 1996;800:138-50.
5. Thompson RW, Parks WC. Role of matrix metalloproteinases in abdominal aortic aneurysms. Ann N Y Acad Sci 1996;800:157-74.
6. Pearce WH, Koch AE. Cellular components and features of immune response in abdominal aortic aneurysms. Ann N Y Acad Sci 1996;800: 175-85.
7. Rao SK, Reddy KV, Cohen JR. Role of serine proteases in aneurysm development. Ann N Y Acad Sci 1996;800:131-7.
8. McMillian WD, Pearce WH. Increased plasma levels of metalloproteinase-9 are associated with abdominal aortic aneurysms. J Vasc Surg 1999;29:122-9.
9. Feigal DW. Problems with endovascular grafts for treatment of abdominal aortic aneurysm (AAA). J Vasc Surg 2001;33:1361-2.
10. Karch LA, Henretta JP, Hodgson KJ, Mattos MA, Ramsey DE, McLafferty RB, et al. Algorithm for the diagnosis and treatment of endoleaks. Am J Surg 1999;178:225-31.
11. Zarins CK, White RA, Fogarty TJ. Aneurysm rupture after endovascular repair using the AneuRx stent graft. J Vasc Surg 2000;31:960-70.
12. Lindholt JS, Vammen s, Fasting H, Henneberg EW, Heickendorff L. The plasma level of matrix metalloproteinase 9 may predict the natural history of small abdominal aortic aneurysms: a preliminary study. Eur J Vasc Endovasc Surg 2000;20:281-5.
13. McMillian WD, Patterson BK, Keen RR, Shively VP, Cipollone M, Pearce WH. In situ localization and quantification of mRNA for 92-kD type IV collagenase and its inhibitor in aneurysmal, occlusive, and normal aorta. Arterioscler Thromb Vasc Biol 1995;15:1139-44.
14. Pyo R, Lee JK, Shipley JM, Curci JA, Mao D, Ziporin SJ, et al. Targeted gene disruption of matrix metalloproteinase-9 (gelatinase B) suppresses development of experimental abdominal aortic aneurysms. J Clin Invest 2000;105:1641-9.
15. Busuttil RW, Rinderbriecht H, Flesher A, Carmack C. Elastase activity: the role of elastase in aortic aneurysm formation. J Surg Res 1982;32: 214-7.
16. Newman KM, Malon AM, Shin RD, Scholes JV, Ramey WG, Tilson MD. Matrix metalloproteinases in abdominal aortic aneurysm: characterization, purification, and their possible sources. Connect Tissue Res 1994;30:265-76.
17. Jean-Claude JM, Newman KM, Li H, Gregory AK, Tilson MD. Possible key role for plasmin in the pathogenesis of abdominal aortic aneurysms. Surgery 1994;116:472-8.
18. Baxter BT, Davis VA, Minion DJ, Wang YP, Lynch TG, McManus BM. Abdominal aortic aneurysms are associated with altered matrix proteins of the nonaneurysmal aortic segments. J Vasc Surg 1994;19:797-802.
19. Thompson RW, Holmes DR, Mertens RA, Liao S, Botney MD, Mecham RP, et al. Production and localization of 92-kilodalton gelatinase in abdominal aortic aneurysms. J Clin Invest 1995;96: 318-26.
20. Hovsepian DM, Ziporin SJ, Sakurai MK, Lee JK, Curci JA, Thompson RW. Elevated plasma levels of matrix metalloproteinase-9 in patients with abdominal aortic aneurysms: a circulating marker of degenerative aneurysm disease. J Vasc Interv Radiol 2000;11:1345-52.
21. Sinha S, Frishman WH. Matrix metalloproteinases and abdominal aortic aneurysms: a potential therapeutic target. J Clin Pharmacol 1998;38: 1077-88.
22. Moore G, Liao S, Curci JA, Starcher BC, Martin RL, Hendricks RT, et al. Suppression of experimental abdominal aortic aneurysms by systemic treatment with a hydroxamate-based matrix metalloproteinase inhibitor (RS 132908). J Vasc Surg 1999;29:522-32.
23. Curci JA, Petrinec D, Liao S, Golub LM, Thompson RW. Pharmacologic expression of experimental abdominal aortic aneurysms: a comparison of doxycycline and four chemically modified tetracyclines. J Vasc Surg 1998;28:1082-93.
24. Curci JA, Mao D, Bohner DG, Allen BT, Rubin BG, Reilly JM, et al. Preoperative treatment with doxycycline reduces aortic wall expression and activation of matrix metalloproteinases in patients with abdominal aortic aneurysms. J Vasc Surg 2000;31:325-42.
25. Petrinec D, Liao S, Holmes DR, Reilly JM, Parks WC, Thompson RW. Doxycycline inhibition of aneurysmal degeneration in an elastase-induced rat model of abdominal aortic aneurysm: preservation of aortic elastin associated with suppressed production of 92 kD gelatinase. J Vasc Surg 1996;23:336-46.
26. Thompson RW, Baxter BT. MMP inhibition in abdominal aortic aneurysms. Rationale for a prospective randomized clinical trial. Ann N Y Acad Sci 1999;878:159-78.
27. McMillian WD, Tamarina NA, Cipollone M, Johnson DA, Parker M, Pearce WH. Size matters: the relationship between MMP-9 expression and aortic diameter. Circulation 1997;96:2228-32.
28. Liao S, Curci JA, Kelley BJ, Sicard GA, Thompson RW. Accelerated replicative senescence of medial smooth muscle cells derived from abdominal aortic aneurysms compared to the adjacent inferior mesenteric artery. J Surg Res 2000;92:85-95.

29. Newman KM, Jean-Claude J, Li H, Scholes JV, Ogata Y, Nagase H, et al. Cellular localization of matrix metalloproteinases in the abdominal aortic aneurysm wall. J Vasc Surg 1994;20:814-20.
30. Freestone T, Turner RJ, Coady A, Higman DJ, Greenhalgh RM, Powell JT. Inflammation and matrix metalloproteinases in the enlarging abdominal aortic aneurysm. Arterioscler Thromb Vasc Biol 1995;15: 1145-51.
31. Newman KM, Ogata Y, Malon AM, Irizarry E, Gandhi RH, Hagase H, et al. Identification of matrix metalloproteinases 3 (Stromelysin-1) and 9 (Gelatinase B) in abdominal aortic aneurysm. Arterioscler Thromb 1994;14:1315-20.
32. Tamarina NA, McMillian WD, Shively VP, Pearce WH. Expression of matrix metalloproteinases and their inhibitors in aneurysms and normal aorta. Surgery 1997;122:264-72.
33. Parodi JC, Ferreira M, Fornari C, Berardi VE, Diez RA. Neutrophil respiratory burst activity and pro- and anti-inflammatory cytokines in AAA surgery: conventional versus endoluminal treatment. J Endovasc Ther 2001;8:114-24.
34. Odegard A, Lundbom J, Myhre HO, Hatlinghus S, Bergh K, Waage A, et al. The inflammatory response following treatment of abdominal aortic aneurysms: a comparison between open surgery and endovascular repair. Eur J Vasc Endovasc Surg 2000;19:536-44.
35. Juvonen J, Surcel HM, Satta J, Teppo AM, Bloigu A, Syrjala H, et al. Elevated circulating levels of inflammatory cytokines in patients with abdominal aortic aneurysm. Arterioscler Thromb Vasc Biol 1997;17: 2843-7.
36. Rohde LE, Arroyo LH, Rifai N, Creager MA, Libby P, Ridker PM, et al. Plasma concentrations of interleukin-6 and abdominal aortic diameter among subjects without aortic dilatation. Arterioscler Thromb Vasc Biol 1999;19:1695-9.

Submitted Sep 27, 2001; accepted Dec 20, 2001.

DISCUSSION

Dr Timothy Baxter (Omaha, Neb). The observations are important because they represent progress in our understanding of aneurysmal disease, having taken basic science observations and applying these to patients. One goal of the basic science work that people have been doing is to identify aneurysm biomarkers. This is an important step in that direction. I have three questions for you.

First of all, can you tell us, or did you calculate, sensitivity and specificity of MMP-9 for endoleaks? In other words, if we have a patient who has MMP-9 levels at 3 to 6 months that do not go down, what would the chances be that that person has an endoleak? Conversely, if their MMP-9 levels went up, what are the chances that they would not have an endoleak?

The second question is, did you look at aneurysm volume as a marker for MMP levels? I know others have seen correlations between aneurysm size and MMP levels, and so the question would be, in addition to diameter, have you considered volume? I assume that in this endograft group you probably have fairly good volumetric measurements available.

My third question is, MMP-9 is a macrophage product that is a general marker of inflammation, so it may not be specific to aneurysmal disease. The question there is, did you look at other factors, such as infection, or other inflammatory disease processes, such as arthritis, that may have influenced MMP levels in these patients?

Dr David Lorelli. In regards to the sensitivity and specificity, we did not specifically calculate it, although you are correct that the numbers are small in the endoleak group. That becomes significant in that the preoperative ranges of these plasma MMP-9 levels vary quite a bit. That is true not only in this study but in two other studies that have been published previously. So, it is really difficult to define a normal plasma MMP-9 level in patients, and that may well relate to the other factors that you talked about, other diseases that may have increased MMP levels, such as rheumatoid arthritis, certain forms of cancer, and other things. The direction of change actually would be a significant finding clinically as we examine patients with endovascular repair postoperatively to determine whether or not endoleak exists.

In regard to the question about volumetric data, we are currently looking back at that information to determine whether or not any of that is significant. There was no correlation with preoperative MMP-9 with aneurysm diameter; however, it is difficult to say whether or not there is an association with the extent of aneurysm or the volume of aneurysm, and that needs to be further addressed.

One thing I might add as we are continuing our progress here on the long-term follow-up is that we have limited 6-month data in the conventional group that continue to show that their levels continue to decrease, and even though we do not have enough numbers currently to make a statistical comparison, it does appear that their levels at 6 months are lower than their preoperative levels. That fits in well with some data that were reported from the Washington University group showing that there is a significant decrease in conventional repair in MMP-9 levels between 6 and 10 months postoperatively.

Dr John Hallett (Orrington, Me). Sticking with conventional repair, why do you think the levels went up? As you began to present this, I thought with the conventional repair they are obviously going to go down fairly quickly. What is the mechanism do you think that takes them up?

Dr Lorelli. We had anticipated that the levels would go down also. That is not what we actually found. It is unclear whether or not the stress of an open repair initiates an acute inflammatory response or upregulates the chronic inflammatory response that is seen in aneurysm patients that causes this peak in MMP-9 levels at 1 month. In addition, the manipulation of the aneurysm with open repair may contribute to that. It is one of the questions that needs to be answered in the near future.

Dr Gilbert Upchurch (Ann Arbor, Mich). I was sitting there thinking about the same thing Dr Hallett was, and I think I somewhat have an answer to his question. I think you are somewhat obligated to prove these patients do not have aneurysms elsewhere and to document for sure that the correlation is between MMP-9 and endoleak exclusion true. I would suggest performing CTs as well as femoropopliteal artery aneurysm scans. We have published a paper that has shown as many as 15% of male patients with hypertension may have femoral and popliteal artery aneurysms, so you have to look for aneurysms elsewhere.

Dr Lorelli. We had three patients with evidence or history of aneurysms besides the infrarenal aorta, but in review of those three patients, there was nothing that stood out as far as their levels of MMP-9 or any other characteristics of those three patients that would separate them from their cohorts.

Dr Bernardo Martinez (Toledo, Ohio). I do not know if I missed this in the presentation, but did you have any distribution of the endoleak patient population, the eight patients, and the MMP? Do you see any MMP tracing according to the type of endoleaks?

Dr Lorelli. The numbers are too small to make a correlation. We had follow-up data available in five patients: four of those had a type 2 endoleak, and one had a type 1 endoleak. Just looking at the numbers, there was no trend that we could see; again, however, the numbers were quite small to make any statistical comparison.